ELECTROPHYSIOLOGICAL MEASUREMENTS OF CARBON DIOXIDE PERCEPTION IN THREE SPECIES OF TICKS (ACARINA: IXODIDAE)

By

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PREFACE

This investigation was designed to determine if certain physiological or environmental factors influence carbon dioxide perception in Amblyomma maculatum Koch, Amblyomma americanum (L.) and Dermacentor variabilis (Say). Actual measurement of carbon dioxide perception was accomplished through use of electrophysiological techniques. The effects of species, sex, age, humidity, temperature and ambient CO_2 concentration on carbon dioxide perception were determined and statistically analyzed.

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CHAPTER I

INTRODUCTION

Tick chemoattraction has received substantial attention among researchers in recent years and much emphasis has been directed towards its possible utilization in tick control programs and population density studies. To date, the majority of work in this area has dealt with using carbon dioxide (CO_2) as the attracting medium. Garcia (1962, 1965, 1969), Nevill (1964) and Miles (1968) utilized CO_2 to trap significant numbers of several different tick species. Wilson et al. (1972) incorporated CO_2 in the form of dry ice to collect all developmental stages of the lone star tick, Amblyomma americanum (L.). A small percentage of marked adults were drawn to the CO_2 source from a maximum distance of 21.3 m.

Laboratory studies by Sauer et al. (1974) on A. americanum further indicated that the intensity of tick responses to CO_2 is dependent on CO_2 concentration. Although these investigations give support to CO_2 being a stimulant or attractant of ticks, results obtained have been inconsistent (Gearhart et al., unpublished).

Indications are that other physiological or environmental factors may play a significant role in tick perception of CO_2. The objective of this study was to determine if differences in species, sex, age, humidity, temperature and ambient CO_2 concentrations had an effect on electrical activity of nerve cells in the Haller's organ in three species of

ixodid ticks.

Evaluation of these parameters was accomplished through electrophysiological techniques. Research results of this type, as they apply to ticks, are sparse. However, work of a somewhat similar nature has been conducted on various species of Diptera (Agee, 1973 and Davis, 1976a, 1976b), Lepidoptera (Baker and Roelofs, 1976), Isoptera (Floyd et al., 1976) and Orthoptera (Washio and Nishino, 1976) and will serve as a reference for experimental designs and techniques in these studies.

CHAPTER II

REVIEW OF LITERATURE

The fact that ticks are capable of sensing and reacting to various chemical stimuli has been researched and documented for many years. LaHille (1905) was the first investigator to postulate that the receptors responsible for olfactory perception are located in Haller's organ. This structure, located on the distal portion of tarsus I of the foreleg, had previously been thought to be auditory in function. In his work LaHille found that normal Boophilus annulatus would not walk across a strip of paper impregnated with Sanol. However, if these particular ticks had their Haller's organs amputated they would not hesitate in their movement across this barrier. Behavioral studies on the sensory perceptions of Argas persicus (Oken) (Hindle and Merriman, 1912), Ixodes ricinus (L.) (Lees, 1948) and Amblyomma hebraeum Koch (Rechav et al., 1977) and electrophysiological studies on the sensory physiology of Ixodes persulcatus P. Sch. (Zolotarev and Elizarov, 1963) have since added support to this original theory of Haller's organ involvement in olfactory perception.

Morphologically, Nuttall (1908) first determined that the Haller's organs of ixodid ticks are comprised of two distinct parts, which he referred to as the anterior pit and posterior capsule. However, it was not until the advent of techniques such as scanning electron microscopy (SEM) that a true understanding of the structure and morphology of this

organ became known. Bruce (1971) utilized SEM to study both the external and internal features of the posterior capsule of Haller's organ in the lone star tick, Amblyomma americanum (L.). Externally be observed a well defined horizontal slit or aperture located in the thin membranous cuticle covering the capsule, with variation occurring between right and left legs as well as between sexes. This concept of external variation in Haller's organ was also observed by Homsher and Sonenshine (1975) in their studies of ten species of ixodid ticks and by Chow and Wang (1975) in investigations of the ixodid ticks Rhipicephalus sanquineus (Latreilla), Amblyomma testudinarium Koch and Boophilus microplus (Canastrini). Internally, Bruce observed several irregularly shaped pleomorphs and cylindrical spines, along with eight symmetrically arranged thin-walled setae. No internal variations were observed between either right or left legs or between sexes.

A more detailed account of the ultrastructure of Haller's organ in A. americanum was conducted by Foelix and Axtell (1972). Their studies, which utilized SEM, included observations of both the anterior pit and posterior capsule and agreed with the findings of Bruce with one exception. Whereas Bruce found eight thin-walled setae in the posterior capsule, Foelix and Axtell could only demonstrate seven.

Scanning electron microscopy studies have also been carried out by Chu-Wang and Axtell (1973) on the claw sensilla of the soft tick Argas arboreus Kaiser, Hoogstraal and Kohls and the hard tick A. americanum and by Waladde (1977) on the cattle tick, B. microplus. They found these sensilla to function not only as mechanoreceptors but as chemoreceptors also.

In her review of the structure of arthropod chemoreceptors, Slifer

(1970) notes that a typical chemoreceptor is composed of (a) cuticular parts, (b) sensory neurons and (c) sheath cells. The cuticular parts are subdivided into thick-walled and thin-walled chemoreceptors, such as those observed by Bruce in the Haller's organ of A. americanum.

Several authors have studied the neural mechanisms which control arthropod behavior and olfactory perception (Erickson, 1963; Huber, 1967; Roeder, 1970; Kaissling, 1971 and Balashov, 1972). In Balashov's work on several species of Ixodidae, he found that ticks have retained a simple bineural (reflex) arc consisting of sensory and motor or association motor neurons from several pairs of glomerular nuclei, which lie within the ganglia of leg I and are connected to Haller's organ. These allow the tick to perceive odors from a considerable distance. Balashov found this particular arrangement to be specific for ixodid ticks.

Behavioral studies by Lees (1948) on the sensory physiology of the sheep tick, I. ricinus, indicate that attraction to a host is due primarily to odors being emitted by the host and temperature stimulation. In regards to temperature, Lees was able to show that ticks are attracted or repelled by changes in air temperature and not by radiant heat. Similiar investigations performed by Wilkinson (1953) on the larvae of the cattle tick, B. microplus, agreed with the findings of Lees. More specifically, on responses to odor, Wilkinson demonstrated that ticks were greatly attracted to a test tube rubbed in human sweat and to cotton swabs which had been rubbed in mucus, saliva and areas of the flank and escutcheon of cattle.

Tick attraction resulting from odors being emitted by the host has also been substantiated by Dethier (1957) in his review of the blood

sucking arthropods and Balashov (1972) in his work with several species of ixodid ticks. Balashov showed that ticks which were released at varying distances from a frequently used host path would orient themselves in close proximity to that path for periods as long as one month after release. He concluded, therefore, that attraction to the path was due to host odor.

Concerning physiological factors which may influence host-seeking behavior, Sactor et al. (1948) calculated the optimum temperature for molting in laboratory reared A. americanum to be 30°C. Relative humidity of 85-100% was also proven to be most favorable for attachment and engorgement. This finding coincides with that of Sauer and Hair (1971) who showed the critical equilibrium humidity (CEH) for A. americanum to be 85% for females and slightly less for males. Hair et al. (1975) made comparisons between the abilities of A. americanum, Amblyomma maculatum Koch and Dermacentor variabilis (Say) to resist dehydration and maintain water balance. Of the three species, A. americanum appeared most dependent on moist microenvironments because of its greater susceptibility to loss of total body water and drop of hemolymph at low humidities.

Although investigations, such as those previously mentioned, have indicated that ticks are attracted to certain volatile chemical odors being emitted by the host, limited attempts have been taken towards the discovery, identification and utilization of these particular compounds. Only carbon dioxide (CO_2) has been investigated to any great degree as a possible tick stimulant for host-seeking behavior.

Garcia (1962, 1965, 1969) utilized CO_2 to trap significant numbers of Ornithodoros coriaceus Koch, Dermacentor occidentalis Marx, Ixodes

pacificus Cooley and Kohls and Dermacentor andersoni Stiles in both laboratory and field tests. Two other members of the genus Ornithodoros have also been collected with the aid of CO_2 (Nevill, 1964 and Miles, 1968). Nevill showed that the sand tampan, Ornithodoros saviayni (Audouin), was capable of detecting a 5% concentration of CO_2 liberated at 1 liter/minute while still below the sand. He therefore concluded that CO_2 in the exhaled breath of the host, ca 4-5% in bovines and humans (Kellogg, 1970), was the main factor causing stimulation and attraction of the sand tampan. Of significance in Nevill's work, however, was the fact that not all of the ticks reacted to the CO_2. Also, of the ticks which were stimulated to move, their reactions were not simultaneous.

Wilson et al. (1972) also used CO_2 incorporated in a dry ice trap to investigate attraction behavior of A. americanum. Although ticks were attracted from as far away as 21.3 m, these CO_2 traps were only capable of collecting 60-80% of the ticks in the trap vicinity. Instead of going directly to the trap, many ticks were observed to take a "waiting attitude" when nearing the trap.

Alternative sources of CO_2, other than dry ice, were utilized by Sauer et al. (1974) to determine what effect CO_2 concentration had on A. americanum. Using commercially purchased cylinders of 100% CO_2 and a Y-tube olfactometer, they concluded that the intensity of the tick response increases with increasing CO_2 concentration up to ca 8%. Some ticks were once again observed to be indifferent to CO_2 stimulation regardless of concentration.

Burris (1974) examined contact and olfactory responses of the lone star tick, A. americanum, to 67 inorganic or organic chemicals soluble

in H_2O or methanol. Those chemicals containing sodium appeared to be most attractive, with statistical analysis showing certain amino acids as the chemicals eliciting the best responses. Burris also performed extensive trials utilizing a 5% concentration of CO_2 in an olfactometer system. Although results suggested an attraction response to CO_2, the averages were lower than the expected efficiency of the olfactometer. Again, questions were raised regarding factors responsible for CO_2 perception in ticks.

The use of electrophysiology as an effective means of investigating the sensory physiology of certain arthropods has been well documented. The slow and summed receptor potentials elicited by odors were first recorded by Schneider (1957) in the antenna of the male silkmoth, Bombyx mori (L.). Since then, this technique has been adapted to a variety of different investigative procedures. Electroretinograms (ERG's) have been used to measure spectral sensitivity in adult bollworms, Heliothis zea (Boddie) and tobacco budworms, Heliothis virescens (F.) (Agee, 1973), Caribbean fruit flies, Anastrepha suspensa (Loew) (Agee and Park, 1975) and screwworm flies, Cochliomyia hominivorax (Coquerel) (Goodenough, 1977). Electroantennogram (EAG) responses to various compounds have been monitored in the cabbage looper, Trichoplusia ni (Hubn) (Mayer, 1973), yellowfever mosquito, Aedes aegypti (L.) (Davis and Rebert, 1972 and Davis, 1976a, 1976b, 1977), Japanese gypsy moth, Porthedria dispar japonica (Yamada et al., 1976), bark beetle, Dendroctonus frontalis Zimm, (Payne and Dickens, 1976), tobacco hornworm, Manduca sexta (Schweitzer et al., 1976), American cockroach, Periplaneta americanum (L.) (Washio and Nishino, 1976), subterranean termite, Reticulitermes lucifugus santonensis (Feytoud) (Floyd et al., 1976) and redbanded

leafroller, Argyrotaenia velutinana (Walker) (Baker and Roelofs, 1976). Even responses of the acoustic sense cells of adult bollworms and tobacco budworms have been examined electrophysiologically (Agee, 1967).

Extensive electrophysiological investigations involving ticks, however, have not been attempted. Gregson (1967) utilized electrograms to monitor the feeding behavior of D. andersoni. He observed many types of traces and associated them with different aspects of the ticks feeding pattern. Sweatman and Gregson (1970) conducted similar feeding electrograms on Hyalomma aegyptium and showed the importance of temperature on the rate of engorgement. In the area of olfactory perception, Zolotarev and Elizarov (1963) studied chemoreception in I. persulcatus. After implanting a microelectrode into the base of Haller's organ and subjecting the tick to a variety of commercially prepared repellents, they established that these chemicals effect the tick by stimulating the contact chemoreceptors in Haller's organ. Further experiments by Elizarov (1964) on Hyalomma asiaticum and I. persulcatus demonstrated that a volley of impulses of varying amplitudes could be elicited 0.5 to 1.0 seconds after introduction of various chemical irritants (odors) into Haller's organ. These volleys were usually observed to be monophasic with negative polarity but would at times be of positive polarity. More recently, Waladde and Rice (1977) obtained characteristic chemoreceptor responses to solutions of sodium chloride, adenosine triphosphate, reduced glutathion and bovine plasma from cheliceral pit sensilla of the cattle tick, B. microplus, by electrophysiological techniques. This work adds a sensory function to the chelicerae of ticks, appendages which are generally regarded as mere cutting tools.

CHAPTER III

MATERIALS AND METHODS

Tick Rearing

Experiments were conducted on adult Gulf Coast ticks, Amblyomma maculatum Koch, lone star ticks, Amblyomma americanum (L.) and American dog ticks, Dermacentor variabilis (Say). Larval and nymphal stages were reared on domestic rabbits at Oklahoma State University and stored in cardboard containers in sealed plexiglass humidity chambers containing saturated salt solutions.

Experimental Design

All ticks were preconditioned a minimum of one week prior to testing in sealed plexiglass humidity chambers. Due to the comparative nature of these studies, specific environmental and physiological conditions under which ticks were preconditioned (i.e. humidity, temperature, ambient CO_2 concentration and age) varied according to the tests performed. A photoperiod of 14 hrs daylight 10 hrs. darkness was kept constant throughout all tests.

Age Study

Ticks of 1-2, 4-6, 8-10, 12-14 and 16-18 weeks post-molt were tested after being preconditioned at room temperature (26 $\pm$ 0.5^{o}C) to a high relative humidity (87 $\pm$ 4%) and low ambient CO_2 concentration

(422 ± 65 ppm). Humidity was kept constant using a saturated solution of K_2SO_4 (Winston and Bates, 1960). Ambient CO_2 concentration was maintained by using a 1/82 hp Fischer® Dyna-pump which circulated fresh air through the chamber. The level of CO_2 was checked daily with an Infrared® Model 755-010 spectrometer in this and subsequent studies.

Ambient CO_2 Study

Ticks of 8-10 weeks post-molt were tested after being preconditioned at 26 ± 0.5°C and 87 ± 4% RH to either a low (422 ± 65 ppm) or high (956 ± 78 ppm) ambient CO_2 concentration. Humidity was maintained with a saturated solution of K_2SO_4 (Winston and Bates, 1960). Low ambient CO_2 concentration was kept constant by using a Fischer® Dyna-pump. High ambient CO_2 levels were obtained by not circulating fresh air through the chamber, thus allowing normal room CO_2 concentrations to build-up within the chamber. Carbon dioxide levels were checked daily.

Humidity Study

Ticks of 8-10 weeks post- molt were tested after being preconditioned at 26 ± 0.5°C and 422 ± 65 ppm CO_2 to either a low (65 ± 5%), medium (74 ± 2%) or high (87 ± 4%) relative humidity. These respective humidity levels were obtained by using saturated solutions of $NaNO_2$, NaCl and K_2SO_4 (Winston and Bates, 1960). Carbon dioxide concentration was maintained using a Fischer® Dyna-pump and checked daily.

Temperature Study

Ticks of 8-10 weeks post-molt were tested after being preconditioned at 87 ± 4% RH and 422 ± 65 ppm CO_2 to either a low (21 ± 0.8°C), medium

(26 ± 0.5°C) or high (32 ± 0.4°C) temperature. The low temperature was maintained by using a standard room air conditioner and the high temperature by using a Titan® Model T760B portable room heater. The medium temperature was equivalent to room temperature where tests were being performed. Humidity was maintained with a saturated solution of K_2SO_4 (Winston and Bates, 1960). Carbon dioxide concentration was kept constant by using a Fischer® Dyna-pump and checked daily.

Experimental Set-up

A copper screen lined Faraday cage served as a holding area and provided an electrically grounded environment for tick preparation and testing. Ticks were immobilized with their ventral side up on double-sided Borden® mystik tape and positioned on a Pyrex® gas dispersion tube situated under a Bausch and Lomb® Model AVB-73 stereomicroscope. The dispersion tube was connected to a commercially prepared cylinder of 350 ± 18 ppm CO_2 and allowed for regulation of ambient CO_2 concentrations during testing.

An insulated (except at the tip) stainless steel microelectrode was inserted into the tick body in a ground position and served as an indifferent electrode. A similar recording microelectrode was inserted through the corium between the metatarus and tarsus just posterior to Haller's organ (located on the dorsal tarsal surface), and connected to the input of a Grass® Model P15 differential AC preamplifier. The amplified (1000x) and filtered nerve impulses were then displayed on a Tektronix® Model 5113 dual beam storage oscilloscope. A Realistic® Model SA-10 solid state stereo amplifier and Realistic® Model Solo-5 stereo speaker were also connected to the preamplifier output and

allowed for auditory observations of tick action potentials.

Administration of CO_2 concentrations used for tick stimulation was accomplished by a fluidic gas delivery system (Pierce, 1976). The system intake was connected to a commercially prepared cylinder of 950 ± 48 ppm CO_2 and the outlet connected in circuit to the gas dispersion tube. This provided a means by which ambient CO_2 concentrations surrounding the tick could be increased from ca 350 ppm to 950 ppm at any time during testing. A 5 ft section of ¼ in Tygon® tubing was connected to an Infrared® Model 755-010 spectrometer and Linear® Model 485 strip chart recorder and positioned directly above the dispersion tube. This enabled CO_2 concentrations to be detected and recorded throughout the test period.

Data Collection and Analysis

Five female and five male ticks of each species were tested from each variable within a particular study. All ticks were purged (stimulated) ten times with a ten second burst of 950 ± 48 ppm CO_2 every two minutes. Prior to CO_2 stimulation a steady baseline was obtained, with few if any nerve impulses being observed. However, at the onset of CO_2 stimulation a rapid volley of impulses was produced which eventually returned to the initial steady baseline. This response time, in seconds, of nerve impulses produced from each burst of increased CO_2 concentration was recorded and mean values calculated.

A standard analysis of variance was run on the data and significance determined by using Duncan's Multiple Range test for comparison of treatment means.

CHAPTER IV

RESULTS

Age Study

The effects of age on mean response times to CO_2 are shown in Table 1. Average response times to CO_2 (ca 950 ppm) in A. maculatum were significantly different ($P < 0.05$) between sexes at all ages tested. Average response times in A. americanum showed significant differences ($P < 0.05$) between sexes at ages 1-2, 8-10 and 12-14 weeks post-molt. However, D. variabilis only showed significant differences ($P < 0.05$) between sexes at age 8-10 weeks post-molt.

Average response times of female ticks of each species to CO_2 (ca 950 ppm) showed A. maculatum to be significantly different ($P < 0.05$) from the other tick species throughout all ages tested. At ages 1-2 and 4-6 weeks post-molt, female response times were significantly different ($P < 0.05$) between all species. However, female response times of A. americanum and D. variabilis at ages 8-10, 12-14 and 16-18 weeks post-molt were not significantly different.

Average response times of male ticks of each species to CO_2 (ca 950 ppm) were not significant at ages 1-2, 4-6 and 8-10 weeks post-molt. At ages 12-14 and 16-18 weeks post-molt, significant differences ($P < 0.05$) in response times were observed between males of A. americanum and the other two tick species.

When one compares mean response times to CO_2 (ca 950 ppm) within

TABLE 1. Mean response time to CO_2 *vs* age in three species of ixodid ticks.

	MEAN RESPONSE TIME (Sec.)*					
	Amblyomma maculatum		*Amblyomma americanum*		*Dermacentor variabilis*	
Age**	♀	♂	♀	♂	♀	♂
1-2	43 b	31 efgh	36 cde	25 hijkl	25 hijkl	29 fghi
4-6	40 bc	28 ghij	31 efgh	26 ghijk	23 jklm	24 ijkl
8-10	53 a	25 hijkl	34 def	22 jklm	32 efg	24 ijkl
12-14	38 bcd	20 klm	25 hijkl	32 efg	29 fghi	24 ijkl
16-18	32 efg	17 m	21 klm	26 hijk	24 ijkl	19 lm

*Means followed by the same letter are not significantly different at the 5% level (Duncan).

**Weeks post-molt

a species and sex at each age, significant differences ($P < 0.05$) are observed. However, these differences are inconsistent and do not follow a particular pattern in all species.

Ambient CO_2 Study

The effects of ambient CO_2 concentration on mean response times to CO_2 are shown in Table 2. In all three species, average response times to CO_2 (ca 950 ppm) at low ambient CO_2 levels (422 ± 65 ppm) were significantly different ($P < 0.05$) between sexes. At high ambient CO_2 levels (956 ± 78 ppm), significant differences ($P < 0.05$) between sexes were observed in A. maculatum and A. americanum.

Average response times of female ticks of each species to CO_2 (ca 950 ppm) at 422 ± 65 ppm ambient CO_2 were significantly different ($P < 0.05$) between A. maculatum and the other two tick species. At 956 ± 78 ppm ambient CO_2, significant differences ($P < 0.05$) were observed between mean response times of female D. variabilis and those of A. maculatum and A. americanum.

Average response times of male ticks of each species to CO_2 (ca 950 ppm) at 422 ± 65 ppm ambient CO_2 were not significant. At 956 ± 78 ppm ambient CO_2, however, significant differences ($P < 0.05$) in male response times were observed between A. americanum and the other two tick species.

Comparison of mean response times to CO_2 (ca 950 ppm) within a species and sex, show significant differences ($P < 0.05$) occurring between 422 ± 65 ppm and 956 ± 78 ppm ambient CO_2 in all cases.

TABLE 2. Mean response time to CO_2 *vs* ambient CO_2 levels in three species of ixodid ticks.

TICK SPECIES	MEAN RESPONSE TIME (Sec.)*			
	High ambient CO_2**		Low ambient CO_2***	
	♀	♂	♀	♂
Amblyomma maculatum	25 c	20 de	53 a	25 c
Amblyomma americanum	24 c	14 f	34 b	22 cd
Dermacentor variabilis	18 e	18 e	32 b	24 c

*Means followed by the same letter are not significantly different at the 5% level (Duncan).

**956 ± 78 ppm

***422 ± 65 ppm

Humidity Study

The effects of humidity on mean response times to CO_2 are shown in Table 3. In all three species, average response times to CO_2 (ca 950 ppm) at the high humidity (87 ± 4%) were significantly different ($P < 0.05$) between sexes. However, only the medium (74 ± 2%) and low (65 ± 5%) humidities revealed significant differences ($P<0.05$) between the sexes of D. variabilis and A. americanum, respectively.

Average response times of female ticks of each species to CO_2 (ca 950 ppm) at 87 ± 4% RH and 74 ± 2% RH were significantly different ($P<0.05$) between A. maculatum and the other two tick species. At 65 ± 5% RH, significant differences ($P<0.05$) were observed between mean response times of female D. variabilis and those of A. americanum and A. maculatum.

Average response times of male ticks of each species to CO_2 (ca 950 ppm) at 87 ± 4% RH were not significantly different. However, at 74 ± 2% RH and 65 ± 5% RH response times of male A. maculatum were significantly different ($P<0.05$) from A. americanum and D. variabilis.

When comparing mean response times to CO_2 (ca 950 ppm) at each humidity within a species and sex, significant differences ($P<0.05$) are observed between 87 ± 4% RH and 65 ± 5% RH in all cases. However, comparison of mean response times at 74 ± 2% RH vs 87 ± 4% or 65 ± 5% RH within a species and sex show significant differences to be inconsistent.

Temperature Study

The effects of temperature on mean response times to CO_2 are shown in Table 4. In all three species, average response times to CO_2 (ca 950 ppm) at the medium temperature (26 ± 0.5° C) were significantly

TABLE 3. Mean response time to CO_2 *vs* humidity in three species of ixodid ticks.

TICK SPECIES	MEAN RESPONSE TIME (Sec.)*					
	High humidity**		Medium humidity***		Low humidity****	
	♀	♂	♀	♂	♀	♂
Amblyomma maculatum	53 a	25 cd	27 c	24 cde	22 efg	20 fg
Amblyomma americanum	34 b	22 defg	23 def	20 fgh	21 efg	17 hi
Dermacentor variabilis	32 b	24 ce	22 def	19 def	15 i	15 i

*Means followed by the same letter are not significantly different at the 5% level (Duncan).

**87 ± 4%

***74 ± 2%

****65 ± 5%

TABLE 4. Mean response times to CO_2 *vs* temperature in three species of ixodid ticks.

TICK SPECIES	MEAN RESPONSE TIME (Sec.)*					
	High temperature**		Medium temperature***		Low temperature****	
	♀	♂	♀	♂	♀	♂
Amblyomma maculatum	41 b	27 d	53 a	25 de	19 h	19 h
Amblyomma americanum	39 b	31 c	34 c	22 fg	23 efg	20 gh
Dermacentor variabilis	25 de	23 ef	32 c	24 ef	28 d	28 d

*Means followed by the same letter are not significantly different at the 5% level (Duncan).

**32 ± 0.4°C

***26 ± 0.5°C

****21 ± 0.8°C

different ($P < 0.05$) between sexes. Average response times to CO_2 (ca 950 ppm) at the low temperature ($21 \pm 0.8^{o}C$) showed no significant differences occurring between sexes. At the high temperature ($32 \pm 0.4^{o}C$) significant differences ($P < 0.05$) in mean response times to CO_2 (ca 950 ppm) were observed between sexes in A. maculatum and A. americanum, but not in D. variabilis.

Average response times of female ticks of each species to CO_2 (ca 950 ppm) at $21 \pm 0.8^{o}C$ were significantly different ($P < 0.05$) in all cases. Average response times of female ticks of each species at $32 \pm 0.4^{o}C$ showed significant differences ($P < 0.05$) between D. variabilis and the other two tick species. At $26 \pm 0.5^{o}C$, significant differences ($P < 0.05$) in response times were observed between females of A. maculatum and those of A. americanum and D. variabilis.

Average response times of male ticks of each species to CO_2 (ca 950 ppm) at $32 \pm 0.4^{o}C$ were significantly different ($P < 0.05$) in all cases. At $26 \pm 0.5^{o}C$, mean response times of male A. maculatum were significantly different ($P < 0.05$) from male A. americanum. Average response times to CO_2 (ca 950 ppm) in males at $21 \pm 0.8^{o}C$ showed significant differences ($P < 0.05$) between D. variabilis and the other two tick species.

When comparing mean response times to CO_2 (ca 950 ppm) at each temperature within a species and sex, significant differences ($P < 0.05$) are observed between $32 \pm 0.4^{o}C$ and $21 \pm 0.8^{o}C$ in A. maculatum and A. americanum, respectively. Comparison of mean response times at $26 \pm 0.5^{o}C$ in A. maculatum and A. americanum showed significant differences to be inconsistent. Significant differences in mean response times were also inconsistent when comparing D. variabilis at each temperature.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Results of this comparative study were interesting in that a wide and varied response was observed from each tick species and between sexes within a species, to the controlled variables. One of the most striking differences in tick response time to CO_2 was observed in 8-10 week post-molt A. maculatum females. The mean response time was 53 seconds, approximately 20 seconds longer than females of the other two species. This increased stimulation to CO_2 may be one factor involved in the selection of a feeding site on cattle, its primary host as an adult tick. This particular tick species is normally found on the ears of cattle, where the concentration of CO_2 is very substantial due to normal respiration. In contrast, A. americanum and D. variabilis do not seem to prefer any single feeding location as evidenced by observations of their natural hosts under field conditions. Differences in mean response times between sexes within a particular species were usually consistent, with female response times being greater than males. This finding agrees with field tests conducted by Wilson (1972) in which greater numbers of female A. americanum ticks were collected from CO_2 baited traps than males.

Another interesting aspect of this study was the effect on tick response time when they were preconditioned to either low or high ambient concentrations of CO_2 prior to testing. When ticks were

conditioned to low ambient CO_2 concentration (ca 400 ppm), the response time was always greater for all tick species and sexes when compared to high preconditioned CO_2 concentration (ca 950 ppm). This suggests that the background CO_2 concentration may be an important factor in the tick's ability to sense and locate a source of CO_2 under field conditions. In the field, normal diurnal CO_2 concentration is approximately 350-450 ppm with nocturnal concentrations reaching 850-950 ppm (Gearhart, unpublished data). Field work by Wilson (1972) on A. americanum showed a 55% retrap of adult ticks during daylight hours, but only a 5% retrap at night. This decrease in trapping efficiency could possibly be explained by our findings as well as other factors such as decreased nocturnal temperatures.

Another parameter measured was the effect of different humidities on tick response time to CO_2. This study was conducted under conditions equivalent to diurnal ambient CO_2 concentrations with a high concentration of CO_2 (ca 950 ppm), simulating a tick trap, being used to stimulate the tick. In all cases, as humidity increased, response time increased within a particular sex and species. In nature, tick survival and longevity is enhanced when the microenvironment humidity is constantly greater than 85% (Hair et al., 1975). Observations of tick (A. americanum) collection in the field, over a period of several years, indicate that the most ideal tick trapping areas are in shaded, leaf littered, forest floor habitats or high grass and brushy ecotone habitats (Wilson, 1972). The microenvironment humidity is normally high in these areas and thus, an ideal habitat for tick survival.

Tick behavior and seasonal activity is also affected by temperature (Wilson, 1972). The purpose of our temperature study was not to

elucidate the high and low temperature activity thresholds, and their effect on tick response to CO_2, but rather, to determine what effect temperature that are most prevalent during the tick activity season might have on tick response time to CO_2. In general, the differences in response times to CO_2 at the different temperatures were affected by the tick species and the sex of ticks of a species. Amblyomma americanum was most responsive to CO_2 at the highest temperature (ca 32°C) whereas, the other two tick species were most responsive to CO_2 at the medium temperature (ca 26°C). Wilson, in his work with A. americanum, found that CO_2 trapping efficiency increased with increasing temperature up to a peak of ca 28° C. These observed differences may, therefore, indicate approximate temperatures at which CO_2 traps would be most effective for tick trapping of a particular species.

Our findings indicate that species, sex, age, ambient CO_2 concentration, humidity and temperature do play a role in tick perception of CO_2. These results may, therefore, lend themselves to future investigations involving trap-retrap studies or tick population density surveys utilizing CO_2 traps. Results may also be of importance in rearing and preconditioning of laboratory ticks prior to use in field studies. As with all electrophysiological investigations of this type, field tests of an identical nature need to be performed before a true understanding of CO_2 perception can be obtained.

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VITA 2

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